REMARKS

Entry of the foregoing and further and favorable consideration of the subject application is respectfully requested and such action is earnestly solicited.

Applicants respectfully request an interview with the Examiner and Supervisor Andrew Wang, once the Examiner has had an opportunity to review the present Reply & Amendment. Given the length of the prosecution of the present application, Applicants wish to expedite the allowance of the present application and believe that an interview will help eliminate any remaining concerns the Examiner may have with the present application.

The summary of the currently pending claims in the Official Action is incorrect. The claims currently pending in the present application are claims 65-97. However, as the Examiner correctly noted on page 12 of the Official Action, claim 65 was added in the amendment filed November 19, 2001. Applicants submitted new claims 65-97 in an amendment filed July 12, 2002; these claims should have been numbered 66-98. Claim 65 submitted on November 19, 2001, has been canceled by the present amendment. New claim 99 is added by the present amendment, as discussed below. Applicants thus respectfully submit that claims 66-99 are currently pending in this case. Applicants respectfully request that the pending claims be renumbered to reflect this. A clean set of the currently pending claims, as renumbered, and with current amendments and added claims, is provided for the Examiner's convenience.

By the present amendment, renumbered claim 66 has been amended to more precisely define the presently claimed invention. Support for the amendment to claim 66

can be found, at least, on page 4, line 28 to page 5, line 27, and in the Examples. The other pending claims have been amended to correspond with the amendments to independent claim 66 or to more precisely define the presently claimed invention. New claim 99 has been added to encompass step (E), which is deleted from renumbered amended claim 66. No new matter is believed to have been added. Applicants expressly reserve the right to file a continuation/divisional application on any subject matter canceled by the present amendment.

Drawing Objections

The drawings stand objected to for various informalities. Applicants file herewith a set of formal drawings correcting the defects identified in the Notice of Draftsperson's Patent Drawing Review. Withdrawal of this objection is respectfully requested.

Information Disclosure Statement

The Examiner notes on page 4 of the Office Action that the references listed in the specification do not form a proper Information Disclosure Statement. Applicants respectfully assert that the references cited in the specification were properly cited in an Information Disclosure Statement (IDS) submitted on November 1, 1999. This IDS was acknowledged as considered in the Office Action issued by Examiner Wessendorf in the Official Action mailed November 7, 2000. Therefore, Applicants believe no further action is required on this matter, and the objection should be withdrawn.

Summary of the Presently Claimed Invention

Applicants respectfully submit that many of the rejections discussed below appear to stem from a misunderstanding of the presently claimed invention. Accordingly, Applicants first wish to summarize the invention in the hope of clarifying the presently claimed invention for the Examiner. Applicants submit herewith two attachments, Appendices A and B, that diagrammatically depict an embodiment of the presently claimed invention. Appendix A (left side) shows the overall outline of the process beginning with the creation of antibody or antibody fragment libraries from various mRNAs and insertion into bacteriophages. The immunoglobulins (Igs) to a specific target structure are selected ("7" in the diagram) via the claimed process resulting in the isolation of bacteriophages which express the antibodies or antibody fragments selected by the process. The phagemid containing the sequence identifying information for the target structure can be amplified in *E. coli* culture to generate specific scFvs to the target structure.

Appendix A (right side) also shows the general cycle of panning methodology. The phage library is added to immobilized antigen (*e.g.*, mounted tissue). Unbound phages are eliminated by washing, and antigen-specific (*i.e.*, target-specific) phages are eluted by *e.g.*, pH shock or enzymatic cleavage. The target-specific phages are used to infect bacteria, and bacterial colonies are grown. Colonies with the phagemid are selected, and large quantities of phages are grown and purified.

Appendix B shows the steps of the process in terms of independent claim 66. Applicants note that the process is not clear-cut. For example, elements may bind to, *e.g.*,

cancer tissue, that are not cancer-specific. Accordingly, the process is repeated a number of times (*i.e.*, iteratively) to purify the phage which are likely to be cancer-specific. Thus, while the process is not a "clean" process, it does allow one to use multiple rounds of selection to identify target-specific antibodies. For example, one may start with 10^{10-13} different phage in the initial library and slowly reduce the numbers to a manageable level by multiple cycles of selection. Thus, claim 66 (step (B)) indicates that if one exposes a "normal" tissue section, *e.g.*, to a library and remove the supernatant, in doing so one removes the phage which are not capable of binding to "normal" tissue. If this supernatant is exposed to another "normal" tissue section and the supernatant is again removed (new claim 99), then one is enriching for those phage which do not bind normal tissue. Again, Applicants note that this is not a clean process, so the first enriched library will have some binders, some weak binders, and some non-binders. Repeating the process enriches for non-binders and further eliminates binders and weak binders. Thus, one enriches a little further for those phage which really do not bind to the target structure.

Alternatively, one could wash away those phage not binding to a normal tissue section, then remove those phage bound to the section by enzymatic cleavage. These phage may be exposed to a normal section again (new claim 99). Those phage which do not bind are removed by washing, and those phage that bind are removed by enzymatic cleavage. This stepwise process reduces the number of different phage in each "library," but enriches for those phage which really bind - that is, it reduces the complexity of the phage population.

Each time one goes around the cycle, there are positive (*i.e.*, binders) and negative (*i.e.*, non-binders and weak binders) elements generated. Depending upon the sort of tissue used and the aim of the study, one might be more interested in those phage that bind or those phage that do not. One of the strengths of the presently claimed invention is that if one wants to find cancer-specific antibodies, it is best to remove those phage in the library that bind normal cells by exposure to a normal tissue section. Then, one can take the negative population (*i.e.*, non-binders) from this step and expose it to a cancerous tissue section, wash away the non-binders, and remove those really bound by chemical elution or enzymatic cleavage. This phage library of cancer-binding phage is regrown in bacteria, then sent through the selection process again. This same selection process can be applied to cancerous v. non-cancerous tissue, arthritic v. non-arthritic tissue, or embryonic v. mature tissue, and so forth.

Applicants thus note that the above describes the positive and negative selection of the presently claimed invention and explains the generation of the various **enriched** libraries, rather than libraries of pure binders or pure non-binders.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 65-94 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite.

A. Claim 65 (now renumbered claim 66) stands rejected for the recitation of "scFv/Fab fragment thereof." The Examiner asserts that it is not clear what fragments are

intended as scFv and Fab are fragments of an antibody. This rejection, to the extent that it may apply to claim 65 as amended, is respectfully traversed.

Applicants respectfully submit that the present claim language is clear, as claim 66 is referring to the scFv and Fab fragments of an antibody. Nevertheless, without conceding to the merits of this rejection and solely in an effort to expedite prosecution, claim 66 has been amended to recite "scFv/Fab antibody fragment." Withdrawal of this rejection is respectfully requested.

B. Claim 65 (now renumbered claim 66) stands rejected as indefinite in the recitation of "target structure."

Without conceding to the merits of this rejection and solely in an effort to expedite prosecution, renumbered claim 66 has been amended to recite that the elements are bound to a target structure (see steps (B) and (E)). Accordingly, withdrawal of this rejection is respectfully requested.

C. Claim 65 (now renumbered claim 66) stands rejected as indefinite as it is allegedly not clear whether the first and second mounted tissue are different from each other. The Examiner further requests clarification as to the difference between the libraries generated by the claimed method. The Examiner further argues that it is not clear whether the mounted tissue contains the target or not.

Applicants respectfully submit that the first and second mounted tissue are denoted as different by the modifiers "first" and "second." The specification is clear that the two mounted sections can cover a broad variety of different tissue sections. *See, e.g.*, page 3, lines 11-21, and page 5, lines 18-25 of the present specification. For example, the two mounted sections may come from the same physiological or pathological stage but from different donors, permitting the removal of idiotype-specific elements. The two tissue sections may come from the same tumor type but at altered stages of tumor development. The two tissue sections may represent two different developmental stages of normal tissue development (*e.g.*, embryonic v. fetal v. newborn, etc.). Nevertheless, without conceding to the merits of this rejection and solely in an effort to expedite prosecution, renumbered claim 66 has been amended to recite "wherein the second mounted tissue represents a different physiological or pathological state than the first mounted tissue."

With regard to whether the mounted tissue contains the target structure or not, Applicants respectfully submit that the specification and the claim language make it clear that the purpose of the panning procedure is to identify a target structure that is present in one mounted tissue but not the other. Panning with one tissue section first, then a second tissue section will result in the identification of a phage that is capable of binding to one section but not the other. As dependent claim 70 demonstrates, this can be confirmed by comparing the binding of the phage to the first mounted tissue to that of the second mounted tissue. The target structure may be present in either the first mounted tissue

section or the second mounted tissue section. Withdrawal of this rejection is respectfully requested.

D. On page 6 of the Office Action (first full paragraph), the Examiner rejects claim 65 as confusing. The Examiner questions whether the bound elements of step (C) comprise the monoclonal antibody to the target and the bound elements are cleaved from the mounted tissue. This rejection, to the extent that it may apply to the claim as amended, is respectfully traversed.

Bound elements (which make up the second and fourth enriched libraries) comprise the monoclonal antibody or scFv/Fab antibody fragment. However, as is clear from the specification, this antibody or antibody fragment is also attached to some sort of sequence-identifying information (*e.g.*, a phage containing the nucleic acid sequence of the antibody or antibody fragment). Once unbound antibodies or antibody fragments are removed by elution, bound elements are then cleaved from the tissue in order to amplify the library. Applicants respectfully submit that renumbered claim 66, as amended, is clear. Withdrawal of this rejection is respectfully requested.

E. On page 6 of the Office Action (second full paragraph), the Examiner rejects claim 65 (now renumbered claim 66) as allegedly confusing. The Examiner asserts that it is unclear how, in step (I), a monoclonal antibody to the target is isolated or identified from the third enriched library which contains only unbound elements to both the first

mounted tissue and the second mounted tissue. This rejection, to the extent that it may apply to this claim as amended, is respectfully traversed.

The first enriched library contains mostly elements that do not bind to the first mounted tissue (at this point in the panning method, it is not known whether they bind to the second mounted tissue). The second enriched library contains mostly elements that bind to the first mounted tissue (at this point in the panning method, it is not known whether they bind to the second mounted tissue). The third enriched library contains mostly elements that bind to the first mounted tissue, but not to the second mounted tissue. The fourth enriched library contains mostly elements that do not bind to the first mounted tissue, but do bind to the second mounted tissue. Thus, Applicants respectfully submit that the language of renumbered claim 66 is clear, particularly in light of the Examples described in the present specification and the summary of the invention provided above. Withdrawal of this rejection is respectfully requested.

F. Claim 69 (now renumbered claim 70) stands rejected as indefinite because of the phrase "the target structure of the first and second mounted tissue." The Examiner argues that this phrase lacks antecedent basis.

Without conceding to merits of this rejection and solely in an effort to expedite prosecution, claim 70 has been amended to recite "a target structure of the first and second mounted tissues." Withdrawal of this rejection is respectfully requested.

G. Claim 69 (now renumbered claim 70) stands rejected as indefinite in the recitation of "binding pattern." The Examiner requests clarification of this term. This rejection, to the extent that it may apply to claim 70, as amended, is respectfully traversed.

Applicants respectfully submit that the meaning of a binding pattern, in light of the specification, is clear. The Examiner's attention is directed to page 15 of the specification, which describes the use of immunohistochemistry to determine the relative binding of the antibody to the target structure. To one skilled in the art, this term refers to the use of immunochemistry to determine the relative expression, or "pattern," of the target structure in the first mounted tissue versus the second mounted tissue. Nevertheless, without conceding to the merits of this rejection and solely in an effort to expedite prosecution, claim 70 has been amended to delete "pattern." Thus, claim 70 recites the comparison of binding of the individual element against the first and second mounted tissues. One skilled in the art would appreciate that such comparison may include the spatial or quantitative distribution of such binding, as demonstrated in the Examples of the present specification. Withdrawal of this rejection is respectfully requested.

H. Claim 91 (now renumbered claim 92) stands rejected for the limitation of "the recovered bound element" as allegedly lacking antecedent basis.

Without conceding to the merits of this rejection and solely in an effort to expedite prosecution, claim 92 has been amended to recite "bound elements recovered in steps (B) or (E)." Withdrawal of this rejection is respectfully requested.

I. Claim 92 (now renumbered claim 93) stands rejected for the recitation of "the coverage [*sic*] occurs" as allegedly lacking antecedent basis.

Without conceding to the merits of this rejection and solely in an effort to expedite prosecution, claim 93 has been amended to recite "the cleaving of steps (B) or (E) occurs." Withdrawal of this rejection is respectfully requested.

J. Claim 93 (now renumbered claim 94) stands rejected for the recitation of "the cleavage" as allegedly lacking antecedent basis.

Without conceding to the merits of this rejection and solely in an effort to expedite prosecution, claim 94 has been amended to recite "the cleaving of steps (B) or (E)." Withdrawal of this rejection is respectfully requested.

K. Claim 95 (now renumbered claim 96) stands rejected for the recitation of "the elution" as allegedly lacking antecedent basis.

Without conceding to the merits of this rejection and solely in an effort to expedite prosecution, claim 96 has been amended to recite "the eluting of steps (B) or (E)." Withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 87-90 (now renumbered claims 88-91) stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking written description. The Examiner asserts that

the specification does not disclose any initial library comprising an amino acid or a nucleic acid sequence that identifies an antibody. Therefore, there is purportedly no written description for "antibody identifying sequence information." This rejection is respectfully traversed.

Applicants respectfully submit that the present specification does provide written description support for this terminology. Applicants believe that this rejection again stems from a failure to grasp the presently claimed invention. During a telephone conversation with Applicants' undersigned representative on April 15, 2003, the Examiner stated that she interpreted the claims as attempting to claim a "tag," and that she did not believe that there was support for such a recitation. The Examiner's attention was pointed to page 7 of the specification, which describes genetic and/or other antibody-identifying information. Further, Applicants' undersigned representative discussed the concept of phage display, wherein the nucleic acid encoding the antibody is the antibody-identifying information, with the Examiner. Thus, the presently claimed invention reflects the fact that the antibody or antibody fragments are "labeled" such that the antibody can be reproduced once it is isolated in the claimed method.

The Examples of the present specification clearly disclose the use of phage cDNA to determine and reproduce the primary structure of the molecule. Additionally, the specification on page 7 describes polysomes or coded beads, *e.g.*, for use with chemical libraries. Accordingly, Applicants respectfully submit that the present specification

provides adequate written description support for the recitation of antibody sequence-identifying information. Withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C. § 102(b)

Claims 65-76, 78-80, 84-86, and 91 (now renumbered claims 66-77, 79-81, 85-87, and 92) stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Cai et al. (*Proc. Natl. Acad. Sci. USA* 93:6280-6285 (1996)). The Examiner argues that Cai et al. disclose a melanoma-specific antibody (V86) cloned from a phage library. The Examiner argues that tissue sections are cut from the frozen tissue cells of melanoma tumors or normal skin and used for histochemistry, following the method steps of cultured cells. The Examiner argues that the frozen tissue was exposed to the phage library, that the unbound phage was washed and the bound phage identified. This rejection is respectfully traversed.

Applicants respectfully submit that the Examiner has mischaracterized the use of tissue sections by Cai et al. (1996). Cai et al. (1996) do not use the tissue sections for panning the antibody or antibody fragment libraries. Rather, Cai et al. (1996) use melanoma cell lines for panning (see page 6281, 1st col., last bridging paragraph). They further use both the cell lines and the tissue sections for performing immunohistochemistry (see page 6281, col. 1, lines 2-3 and col. 2, "ELISA Assays") to confirm the specificity of the V86 antibody identified during the panning method.

The Cai et al. (1996) publication cited by the Examiner describes the panning of the light chains of and the characterization of antibody V86 that was previously identified in a

panning procedure using immortalized cell lines (Cai et al., *Proc. Natl. Acad. Sci. USA* 92:6537-6541 (1995)). Although Cai et al. (1996) describe a panning procedure, it is directed only towards screening the light chains of the V86 antibodies. Applicants respectfully submit that the method of the presently claimed invention is fundamentally different from that described in either Cai et al. (1996) or Cai et al. (1995).

Cai et al. (1995 and 1996) grow cell cultures and pan in tissue flasks, not against **mounted tissue** as in the presently claimed invention. Accordingly, Cai et al. can only disclose the detection of antibodies to external cell antigens. In contrast, the presently claimed invention possesses the ability to isolate phage to any antigen (extracellular, intracellular, intranuclear, etc.) as long as it is present in the tissue section. *See* page 2, lines 18-25 of the present specification. Applicants note that perhaps no more than 1/10 of the cell's protein is within the membrane, and therefore available for binding in the method of Cai et al. *See* attached abstract of D.M. Brunette and J.E. Till, *J. Membrane Biol.* 5:215 (1971). In contrast, the approach of the presently claimed invention exposes the entire cell antigen portfolio. Thus, Cai et al. lacks an integral element of the presently claimed invention.

Moreover, Cai et al. absorb only once against normal tissue to remove those phage reactive against normal cell surface antigens (see text of Table 2 of Cai et al., 1995), while the presently claimed invention permits the alternating use of normal and altered tissue to greatly enhance the detection of rare phage. Cai et al. (1995 and 1996) do not teach the differential use of mounted tissue sections to enhance the detection of specific targets as

claimed in independent claim 66. Further, with regard to dependent claims 94 and 95, Cai et al. (1995) elute using E buffer, not via enzymatic cleavage as in the presently claimed invention.

Applicants respectfully submit that there is a significant difference between using cell line cultures for panning versus primary tissue. First, a cell line is not a reiteration of the tumor itself in every sense, so antigens expressed on the surface of a cell line will be very different to those in the primary tumor. The use of cell lines far more frequently detects artifacts. Depending upon where the tumor cells are selected from to generate the immortalized cell line, the phenotype of the cells selected may not be representative of other cells in the tumor. For instance, the cells selected for immortalization may be differently responsive to trypsin and/or have different nutritional requirements. Cell lines only represent one sort of cell within a tumor. All tumors thus far described are a heterogeneous population, with various karyotypes (sometimes seen in a cell line) and lineages (*e.g.*, connective, epithelial, haematological tissues), that are not promulgated in a cell line. The cell lines used in Cai et al. (1995) are clones of one line and do not represent the amalgam of cells present in various states of differentiation in a tumor. For example, the Examiner's attention is directed to the classification of phage types on page 6541, lines 6-15, of Cai et al. (1995), wherein only one of the three classes was melanoma-specific, one was tumor-specific (but not melanoma-specific), and one was lineage-specific. With the presently claimed invention, one would not detect this second category of phage and most likely, neither the third. The presently claimed invention enables one skilled in

the art to detect antigens that are diagnostic of the altered tissue or may be even causative to the alteration process. Thus, the presently claimed invention provides a significant advantage over the method disclosed in Cai et al.

Additionally, there are significant technical difficulties that need to be overcome in order to use tissue sections as in the presently claimed invention as opposed to cell lines as disclosed by Cai et al. First, antibodies bind non-specifically to the cell surface via the Fc receptor. Blocking of this reaction using antibodies to CD32 and CD16, e.g., allows specific interactions to occur. Panning after such a blocking step allows for specific interactions to be detected when using a cell line. There is no such mechanism for sections, thus, the first obstacle is to eliminate non-specific interactions. Example 1 demonstrates data regarding non-specific binding for tissue sections. Below 10^9 phage per slide, non-specific binding increased 10-fold. Thus, non-specific binding is a technical difficulty that must be overcome with tissue sections versus cell lines. Second, the panning method described by Cai et al. used cells fixed by a single wash in glutaraldehyde., a common method. Tissue sectioning may proceed in any number of forms, mounted or held in a preserving balm such as paraffin, rapidly frozen and sectioned. The optimal fixation method must be determined for each of these processes, *e.g.*, paraffin embedded tissue is very stable but antigen exposure is adversely affected. Cryostat sections are more representative of the cell but are not easily handled. The present inventors determined whether all or any of these sections were usable within the presently claimed method and then determined the optimal fixation procedure for tissue sections. The disclosure of Cai

et al. in no way aided this process. Third, the tissue section used vastly affects the purification. Example 4 of the present specification shows that when the percent of antigen-positive cells are reduced in the section, a 4-fold decrease in phage binding was observed. Whole cell panning would not be expected to cause such a dilemma. Finally, the panning itself was performed by Cai et al. in a tissue culture flask making the exposure, agitation, and elution mechanically quite easy. The procedure, when "miniaturized" to be performed on a mounted section on a glass slide required considerable adaptation (e.g., placement of the slide in a 50 mL Falcon tube to facilitate effective washing (*see* page 9, line 7-8 of the present specification)). Thus, significant technical difficulties, specific to the use of tissue sections as opposed to cell cultures, were overcome by the inventors in developing the presently claimed invention. Accordingly, Applicants respectfully submit that the Examiner's assertion that Cai et al. anticipate the presently claimed invention is not well-founded.

Because neither Cai et al. (1995 and 1996) reference discloses or suggests each and every element of the presently claimed invention, neither reference can anticipate the presently claimed invention. Additionally, the presently claimed invention provides significant advantages over the methods of Cai et al. (1995 and 1996). Withdrawal of this rejection is respectfully requested.

Conclusion

From the foregoing, further and favorable consideration of the subject application in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

If there are any questions concerning this amendment, or the application in general, the Examiner is respectfully requested to telephone the undersigned.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: June 25, 2003

By: Jennifer Topmiller
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MARKED-UP COPY OF CLAIMS

66. (Twice Amended) A method to acquire a monoclonal antibody or scFv/Fab [fragments thereof] antibody fragment against a target structure comprising the steps of:

(A) exposing a first mounted tissue to an initial antibody library or scFv/Fab antibody fragment library;

(B) eluting directly from the first mounted tissue unbound elements, wherein the unbound elements comprise a first enriched library; and/or

[(C)] recovering a second enriched library comprising bound elements by cleaving the bound elements from a target structure in the first mounted tissue such that a monoclonal antibody or scFv/Fab antibody fragment [thereof] remains bound to the first mounted tissue;

[(D)] (C) amplifying either the first or second enriched libraries;

[(E) repeating steps (A) to (B) to negatively enrich the unbound elements of the first enriched library or repeating steps (A) to (C) to positively enrich the bound elements of the second enriched library;]

[(F)] (D) exposing the [negatively or positively enriched elements of step (E)] the first or second enriched libraries to a second mounted tissue, wherein the second mounted tissue represents a different physiological or pathological state than the first mounted tissue;

[(G)] (E) eluting directly from the second mounted tissue unbound elements from the second mounted tissue, wherein the unbound elements comprise a third enriched library; and/or

[(H)] recovering a fourth enriched library comprising elements bound to the second mounted tissue section [bound elements] by cleaving the bound elements from a target structure in the second mounted tissue such that the monoclonal antibody or scFv/Fab antibody fragment [thereof] remains bound to the second mounted tissue;

(F) amplifying either the third or fourth enriched libraries; and

[(I)] (G) isolating an individual element from either the third or fourth enriched libraries, wherein the individual element is the monoclonal antibody or the scFv/Fab antibody fragment [thereof].

67. (Amended) The method of claim [65] 66 further comprising repeating steps (A) to [(I)] (G).

68. (Amended) The method of claim [65] 66 further comprising repeating steps [(F)] (D) to [(I)] (G).

69. (Amended) The method of claim [65] 66 further comprising repeating steps (A) to [(E)] (C).

70. (Amended) The method of claim [65] 66 further comprising the step of [characterizing] comparing [a] binding [pattern] of the individual element against the target structure of the first and second mounted tissues.

71. (Amended) The method of claim [69] 70, wherein the binding [pattern] is specific for a physiological process.

72. (Amended) The method of claim [70] 71, wherein the physiological process is a pathological process, cell development and differentiation, tissue development and differentiation, a drug response, or a naturally occurring degradation process.

73. (Amended) The method of claim [71] 72, wherein the pathological process is inflammation, a secondary tumor deposit or tumor vasculature.

74. (Amended) The method of claim [65] 66, wherein the target structure of the first and second mounted tissues [tissue sections] is extracellular or intracellular.

75. (Amended) The method of claim [73] 74, wherein the intracellular target structure is located intranuclear of a nuclear membrane.

76. (Amended) The method of claim [73] 74, wherein the extracellular target structure is on the cell surface or a molecule released from a cell.

77. (Amended) The method of claim [75] 76, wherein the cell is a tumor cell.

78. (Amended) The method of claim [75] 76, wherein the molecule is released actively or passively.

79. (Amended) The method of claim [69] 70, wherein the target structure is a ligand, a receptor, an adhesion molecule, a matrix associated molecule or a combination thereof.

80. (Amended) The method of claim [69] 70, wherein the target structure is a protein, a carbohydrate, a nucleic acid or a lipid.

81. (Amended) The method of claim [65] 66, wherein the first or second mounted tissue is a frozen tissue section or a fixed tissue section.

82. (Amended) The method of claim [65] 66, wherein the first or second mounted tissue is pretreated with an enzyme or a chemical.

83. (Amended) The method of claim [81] 82, wherein the enzyme pre-treatment is performed with a protease, a polysaccharase, a ribonuclease, a nuclease or a combination thereof.

84. (Amended) The method of claim [65] 66, wherein the tissue is bone marrow cells, lymph cells, sperm cells or cells from cerebrospinal fluid.

85. (Amended) The method of claim [65] 66, wherein the initial library is a combinatorial library.

86. (Amended) The method of claim [84] 85, wherein the combinatorial library is a naive antibody library, a synthetic antibody library, a semi-synthetic antibody library, or a combinatorial library produced by immunizing against one or more target structures.

87. (Amended) The method of claim [65] 66, wherein step [(D)] (C) of claim 1 comprises amplifying the bound or unbound elements using bacterial cells, PCR synthesis or chemical synthesis.

88. (Amended) The method of claim [65] 66, wherein the monoclonal antibody or scFv/Fab [fragments thereof] antibody fragment of the initial library further comprises antibody identifying sequence information.

89. (Amended) The method of claim [87] 88, wherein the sequence identifying information is a nucleic acid or a amino acid sequence.

90. (Amended) The method of claim [87] 88, wherein the sequence identifying information is in a filamentous phage or a virus.

91. (Amended) The method of claim [89] 90, wherein the filamentous phage is M13.

92. (Amended) The method of claim [65] 66, wherein the [recovered] bound [element] elements recovered [of] in steps [(C) and (I)] (B) or (E) comprise a phage and maintain amplification ability.

93. (Amended) The method of claim [65] 66, wherein the [cleavage] cleaving of steps (B) or (E) occurs between minor coat protein pIII and the monoclonal antibody or scFV/Fab antibody fragment [thereof].

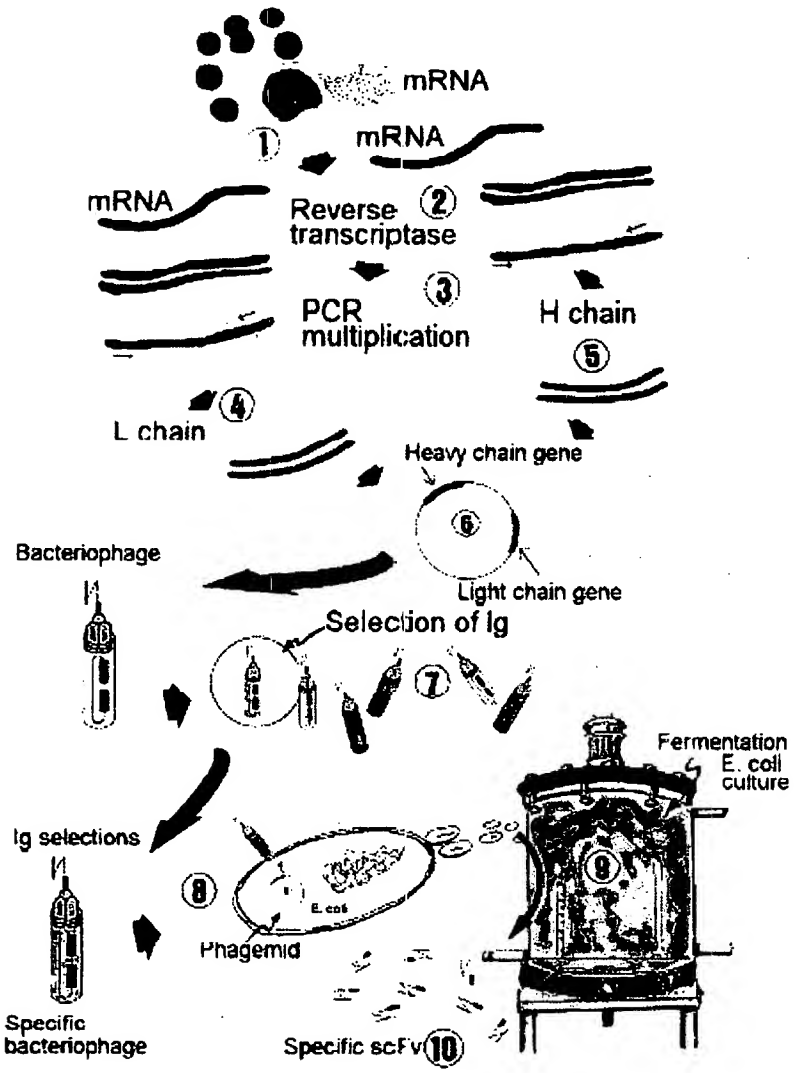
94. (Amended) The method of claim [65] 66, wherein the [cleavage] cleaving of steps (B) or (E) is a proteolytic cleavage and occurs at a protease recognition site.

95. (Amended) The method of claim [93] 94, wherein the proteolytic cleavage [is a proteolytic cleavage and the protease] is performed by Ala64-subtilisin or blood clotting factor Xa.

96. (Amended) The method of claim [65] 66, wherein the [elution] eluting of steps (B) or (E) is a chemical elution.

97. (Amended) The method of claim [95] 96, wherein the chemical elution is an acid or alkaline elution.

98. (Amended) The method of claim [96] 97, wherein the alkaline elution is triethylamine elution.



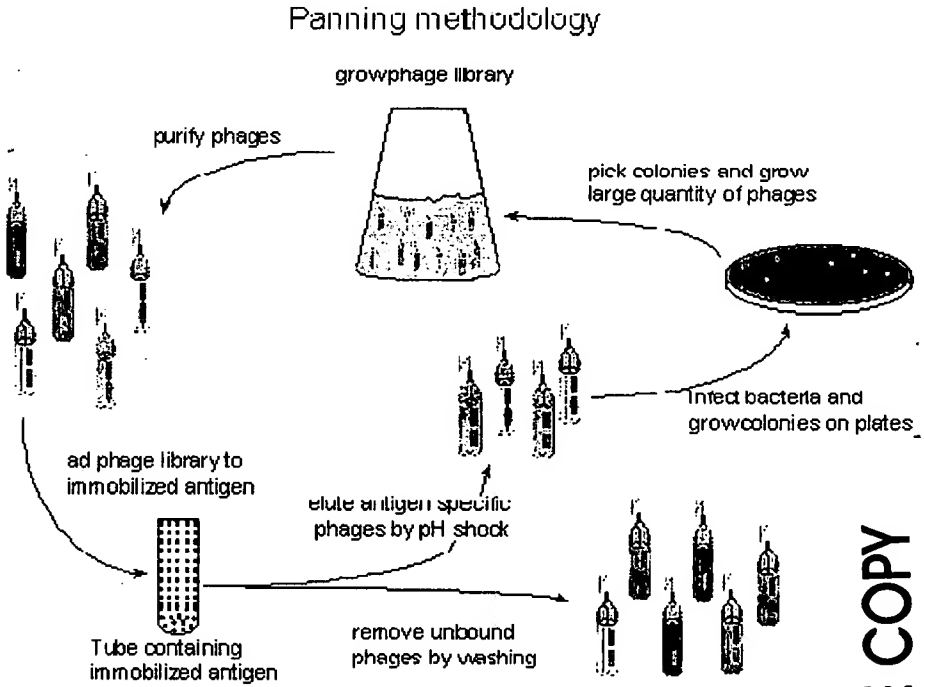


EXHIBIT A

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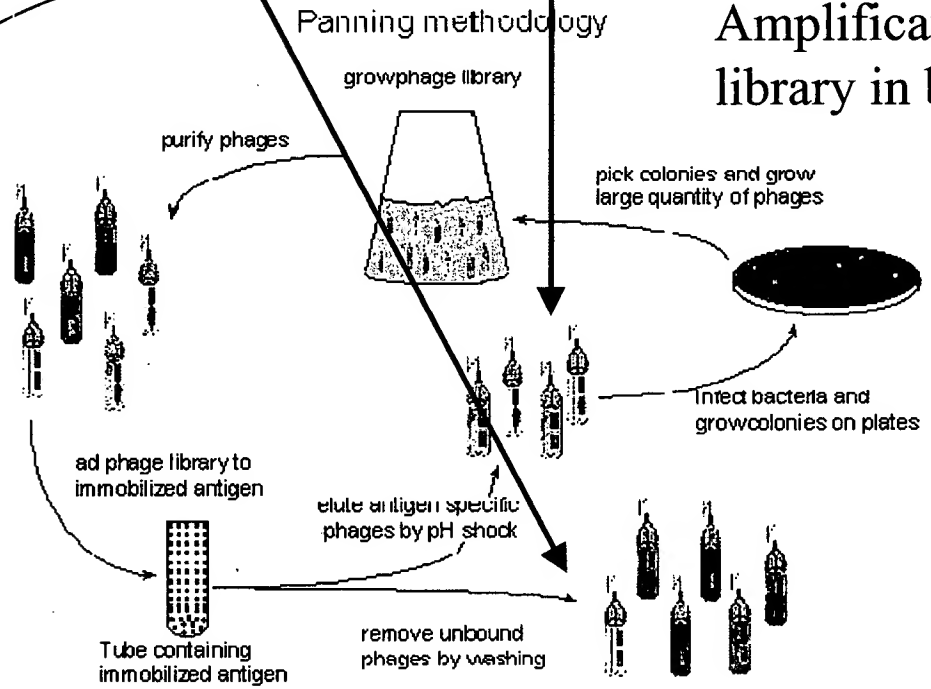
Atty Dkt.: 003300-581
USSN: 09/365,241

This cycle can be repeated again, but using a different tissue section Claim 66 (D), leads to third enriched library isolated here
and fourth enriched library isolated here, Claim 66 (E).

Cycle usually repeated 5-7 times



First Library

Amplification of either library in bacteria

Second Enriched Library Claim 66 (B)

First enriched Library Claim 66(B)

Initial exposure to a tissue section representing *e.g.* cancerous tissue, in the next cycle use non-cancerous tissue section

EXHIBIT B

RPN

J. Membrane Biol. 5, 215–224 (1971)

A Rapid Method for the Isolation of L-Cell Surface Membranes Using an Aqueous Two-Phase Polymer System

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Received 5 February 1970

Summary. A dextran-polyethylene glycol aqueous two-phase system has been used to separate cell surface membranes from other cellular organelles. The surface membranes have been identified on the basis of morphology, content of Na^+, K^+-ATPase, and presence of surface antigen as detected by a ^{51}Cr release method. Contamination of the surface membrane preparations by smooth endoplasmic reticulum, mitochondria, and nuclei has been found to be minimal. An average of 6.5% of the total protein was found in the membrane fraction. Less than two hours is required to isolate the membrane fraction after preparation of a Dounce homogenate. Fractionation by aqueous two-phase polymer systems appears to be a rapid and effective method for the isolation of surface membranes.

A number of methods for the isolation of mammalian cell surface membranes have been described (for reviews *see* Warren, Glick & Nass, 1967; Korn, 1969). Almost exclusively, these methods rely on differences in density or sedimentation characteristics to achieve separation of the surface membranes from other cell organelles. These methods do not always result in satisfactory yields or purity of the isolated material (Wallach, 1967). As the various cellular membranes are known to differ in chemical composition (Bosmann, Hagopian & Eylar, 1968), it is possible that their surface properties reflect these differences. Thus, a technique that separates cellular fractions on this basis would be of some value. The aqueous two-phase polymer systems developed by Albertsson (1960) have the characteristic of separating particles on the basis of differences in their surface properties. In this paper, we present a method for the rapid isolation of L-cell surface membranes in good purity and high yield using an aqueous two-phase dextran-polyethylene glycol system.

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